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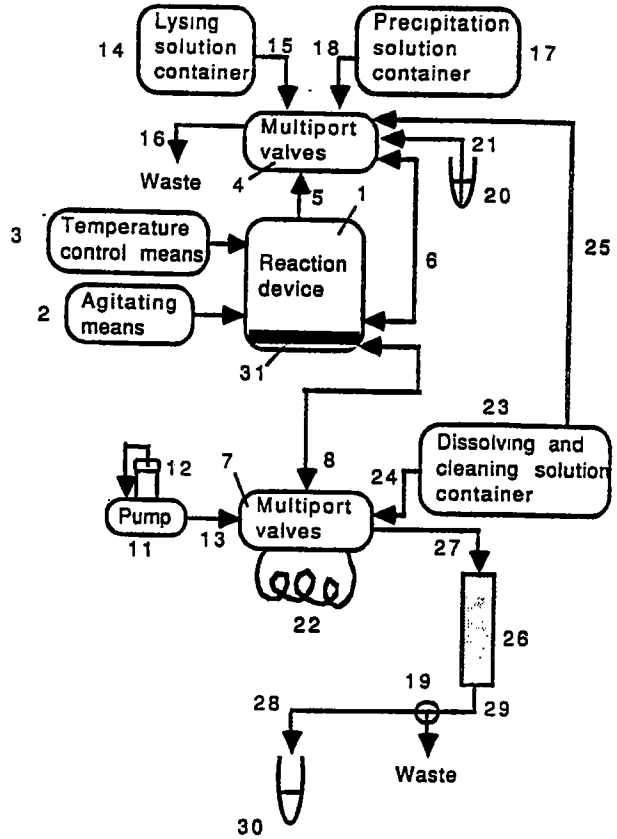
(54) Title: APPARATUS AND METHOD FOR AUTOMATED PURIFICATION OF EXTRA-CHROMOSOMAL DNA FROM A CELL SUSPENSION USING A REACTION DEVICE

(57) Abstract

The invention relates to an apparatus and method for automatic purification of extra-chromosomal DNA from a cell suspension using a reaction device (1) and comprising: means (36, 21, 4, 5, 6, 34, 39) for introducing the cell suspension from a container (20) into a reaction and/or mixing chamber (33), means (2) for agitating the contents of the chamber (33), means (36, 14, 15, 4, 6, 39) for introducing a lysing solution into the chamber (33) to lyse the cells therein, means (36, 17, 18, 4, 6, 39) for introducing a precipitating solution into the chamber (33) to precipitate chromosomal DNA and, optionally, proteins and cell debris therein, means (36, 7, 8, 43) for feeding the liquid contents of the chamber (33) through a filter (31) to a collecting device (22), means (26) for purifying extra-chromosomal DNA from the contents of the collecting device (22), means (36, 25, 23, 24, 4, 5, 7, 8, 43) for introducing a dissolving solution into the chamber (33) to dissolve the precipitate remaining therein, and means (5, 6, 39, 34, 4, 16) for feeding the dissolved precipitate to waste.



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Apparatus and method for automated purification of extra-chromosomal DNA from a cell suspension using a reaction device.

The invention relates to an apparatus and a method for automatic purification of extra-chromosomal DNA from a cell suspension using a reaction device.

Reference is directed to PCT SE89/00144 belonging to the same applicants which is also concerned with an automated method for purification of extra-chromosomal DNA. The contents of the previous application are incorporated herein by reference.

It is known from Birnboim and Doly (Nucl Acids Res 7, 1513-1523, 1979) that plasmid DNA can be purified from Escherichia coli. The bacterial cells are first lysed in an alkaline medium containing a detergent. This is followed by a neutralizing step which yields the plasmid DNA in soluble form while most proteins, cell debris and chromosomal DNA precipitate. Plasmid DNA is then recovered from the mixture by centrifugation and a pure plasmid fraction is obtained by subsequent purification. Kieser (Plasmid 12, 19-36, 1984) has described a similar method, involving a combination of heat treatment and alkali treatment, which gave improved purification of plasmid DNA and less contaminating RNA. In these known methods, the purification involves several separate steps, such as for instance extraction and centrifugation, thus requiring considerable investments in equipment and operator time.

An automated plasmid purification work station based on a laboratory robot has been described by de Bonville and Riedel (Advances in Laboratory Automation Robotics, 1986, ed. Strimatis and Hawk, Zymark Corp., Hopkinton, Ma., pp353-360). That system is based on a number of laboratory unit operations and the robot uses several hardware stations, including a centrifuge, pipettes, capping devices, sample tubes, tip racks, a microtiterplate etc.

Although this work station is automated the system has several disadvantages. Firstly the system has to be set up with tip racks, sample tubes, supernatant tubes etc., which is labour intensive. Secondly, a considerable amount of disposable material is used which increases the expenses for each plasmid purified by the system. Thirdly, several mechanical operations are involved and this might cause technical problems in routine use.

An automated nucleic acid extractor has been described (EP-0 245 945) for extracting and purifying nucleic acids from cells without the use of centrifugation. In the method (described in EP-0 215 533) a lysate is created and mixed with a phenol-based solvent system and then heated to promote phase separation. The lower organic phase is removed and the upper aqueous phase is repeatedly extracted. The aqueous phase is finally dialysed to further purify the nucleic acid solution. The method is used for extraction of high molecular weight DNA (about 10^8 Daltons) from eukaryotic cells such as blood lymphocytes and liver cells. Thus, the method is well suited for extraction of chromosomal DNA from cells.

With reference to PCT SE89/00144, an apparatus and a method for purification of extra-chromosomal DNA from bacteria has been described, which is rapid and avoids phenol extractions and ethanol precipitation. This was attained by a method comprising the following steps:

- introducing the bacterial cell suspension into a chamber,
- introducing a lysing solution into the chamber and mixing it with the contents thereof to lyse the cells therein,
- introducing a precipitating solution into the chamber and mixing it with the contents thereof to precipitate chromosomal DNA and, optionally, proteins and cell debris therein,
- filtering the contents of the chamber through a filter and feeding the liquid contents to a collecting device,
- purifying extra-chromosomal DNA from the contents of the collecting device,

- introducing a dissolving solution into the chamber and mixing it with the contents thereof to dissolve the precipitate remaining therein, and
- feeding the dissolved precipitate from the chamber to waste.

Using this method, a complete cycle for introducing the cell suspension into the chamber to complete regeneration of the chamber and the column took less than ten minutes. The complete purification involving ten samples therefore took approximately ninety minutes under which no supervision or manual labour was needed. The plasmid DNA purified in this way was directly used for restriction analysis and the degree of contamination between different preparations was less than 0.1 percent.

However, the apparatus and the method described have the disadvantages that vacuum and pressurized air is needed for the transport of the cells and to move liquids into and out of the mixing chamber. In addition, the volume of the solutions taken from the solution containers and the volume of the purified DNA solution are determined by the viscosity, time, pressure and/or filter capability. This introduces difficulties to control the operations in a reproducible manner and makes it necessary to have safety margins which means that it is difficult to optimize the yield of DNA as well as the time needed for the different operations.

The main object of the present invention is to provide a reaction device consisting of a reaction chamber with a volume determined by the movement of a piston, which allows the size of the reaction chamber to be controlled by a control unit interfaced with a stepping motor. The piston movement provides the pressure and vacuum needed for the operation according to the invention and the vacuum pump and the pressure air source can be omitted. Solutions and cells to be lysed are introduced into the reaction chamber through a negative pressure obtained by upward movement of the piston. Filtering is achieved by creating a pressure through down-

ward movement with the piston. Relevant valves must be open or closed during these procedures.

The advantage with the apparatus containing the reaction device as compared to the apparatus described in PCT SE89/00144, includes defined sample volumes and defined times for the different operations, which means that less amount of buffers are needed for each cycle and that the cycle time can be further optimized. Also, higher yields are obtained as no safety volumes are needed and the lack of air pressure and vacuum means gives safer handling as no containers need to be pressurized. In addition, the volume of the buffers and the cell suspension can be determined by the movement of the piston, which gives a flexible system easily adapted to different host cells.

The reaction chamber and the accompanying apparatus and method according to the invention will be described more in detail below with reference to the accompanying drawings on which

Fig 1 shows a schematic block diagram of an embodiment of an apparatus according to the invention,

Fig 2 shows a drawing of the reaction device consisting of a chamber with a size determined by the position of a movable piston, and

Fig 3 shows an example of the movement of the piston in the reaction device during a purification and regeneration cycle.

As can be seen from Fig. 2, a reaction and/or mixing chamber 33 with a volume determined by the position of a movable piston 36 is illustrated, comprising a filter with filter support and sealing gasket 31, a stepping motor 32, such as Berger Lahr RPM 564/50, a reaction chamber 33 containing a magnet, capillary tubings 34, 39 and 44, inlet 38 and outlet 37 for cooling and heating water, an O-ring 35, a piston 36, a water jacket for heating/cooling liquid 40, a piston rod with thread 41 and a removable bottom part 42.

The reaction device operates as follows to automatically purify extra chromosomal DNA from a cell suspension with the numbers refering to the schematic drawing of the complete apparatus in Fig.1 (numbers 1-31) and to the reaction device based on a movable piston, see Fig.2 (numbers 31-43). Note that the filter 31 is shown in Figs 1 and 2 and that the capillary tubings 34, 39 and 43 in Fig. 2 represent the tubings 5, 6 and 8, respectively, in Fig. 1. The pressure and sucking of liquids in and out of the mixing chamber 33 is obtained through downward and upward, respectively, movement of the piston 36, through the action of the stepping motor 32. An example of the motion of the piston is shown in Fig. 3 and will be referred to in the text.

The reaction device 1 is provided with agitating means 2, preferably by said magnet, situated in the reaction chamber 33 between the piston 36 and the filter 31, which is acted on by a magnetic stirrer placed under the reaction device 1 (not shown). The magnet motion can be turn on or off through the control of a control unit. The reaction device 1 is provided with means 3 to control the temperature in the reaction chamber 33. The temperature control can preferably be provided by supplying heating/cooling liquids to a space jacket 40 surrounding the reaction chamber 33. The piston 36 of the reaction device 1 is connected to a stepping motor 32 by a piston rod 41 with a thread, which transfers a rotary movement to the up- and downward motion of the the piston 36. The volume of the reaction chamber 33 is determined by the position of the piston. The reaction chamber 33 may be provided with temperature sensor means (not shown) to sense the temperature inside the reaction chamber 33.

At the bottom of the reaction chamber 33, a filter 31 is provided. This filter may consist of material such as metal or polymer.

An inlet/outlet port is connected to an outlet/inlet port of multiport valves 4 via a tubing 39 (6) , while an outlet port in the upper part of the reaction chamber is connected

via a tubing 34 (5) to an inlet port of the multiport valves 4. A pressure valve may be provided to tubing 34 (5) to prevent high pressures in the reaction chamber 33 to be built up during filtration. An outlet/inlet port at the bottom of the reaction device 1 is connected to an inlet/outlet port of the multiport valves 7 via a tubing 43 (8).

The multiport valves 4 exhibit an outlet port connected to waste via a tubing 16. Moreover, the multiport valves 4 is connected to a precipitating solution container 17 via a tubing 18. Another inlet port of the multiport valves 4 is connected to a lysing solution container 14 via a tubing 15.

A tube or vial 20 containing a cell suspension from which extra-chromosomal DNA is to be purified may be connected to an inlet port of the multiport valves 4 via an automatic injector or sampler 21, which is just schematically indicated. It is to be understood that there can be a number of vials 20 from which extra-chromosomal DNA is to be purified in sequential order.

A sample is sucked into the reaction chamber 33 through upward movement of the piston 36 using the step motor 32. The volume of cell suspension inserted into the chamber 33 is determined by the movement of the piston 36 (see Fig. 3).

The multiport valves 7 exhibit an inlet port and an outlet port between which a collecting device in the form of a sample loop 22 of predetermined volume is connected in the embodiment shown. A container 23 containing a dissolving and cleaning solution is connected to an inlet port of the multiport valves 4 and 7 via tubings 25 and 24, respectively.

The inlet end of a chromatography gel filtration column 26 is connected to an outlet port of the multiport valves 7 via a tubing 27, while the outlet end of the column 26 is connected to a drip nozzle 28 of a fraction collector (not shown) via a tubing 29. Drops of purified extra-chromosomal

DNA from the drip nozzle 28 are collected in a vial 30 of the fraction collector. It is to be understood that there can be a number of vials 30 to collect extra-chromosomal DNA extracted in sequence from the cell suspension vials 20.

In the tubings between the outlet end of the column 26 and the drip nozzle 28 of the fraction collector (not shown), a two-way valve 19 is inserted to connect the outlet end of the column 26 to either the drip nozzle 28 or waste.

An eluant for the chromatography column 26 is supplied by a pump 11 from an eluant container 12 via a tubing 13 to an inlet port of the multiport valves 7.

The multiport valves 4 and 7 as well as the agitating means 2, the temperature control means 3, the step motor 32, the sampler 21, the pump 11 and the fraction collector (symbolized by 28 and 30) are all controlled by a control unit (not shown) in a manner known per se.

The apparatus shown on the drawing (Fig. 1) having the reaction device shown in Fig. 2 operates as follows to automatically purify extra-chromosomal DNA from a cell suspension in the vial 20:

The cell suspension is sucked from the vial 20 into the reaction and/or mixing chamber 33 using an upward movement of the piston 36 as schematically shown in Fig. 3 (A). Thus, a predetermined volume of the cell suspension is sucked up by the sampler 21 and enters the mixing chamber through the tubing 6 (39) via the multiport valves 4.

The cell suspension may, optionally, be concentrated by filtering the suspension through the filter 31 (Fig.2) through downward movement of piston 36 as schematically illustrated in Fig.3 (B). The reaction chamber is, optionally, agitated during the filtration by means of the magnet placed in the chamber 33.

The cells in the concentrated cell suspension are, then, lysed in that a predetermined amount of lysing solution from the lysing solution container 14, is introduced into the reaction chamber 33 using upward movement of the piston 36 (Fig.3, C) via the tubing 15, the multiport valves 4 and the tubing 6 (39). The mixture is mixed using the agitating means 2. Optionally, the reaction chamber is heated by means of the temperature control means 3, which is achieved by introducing preheated liquid into the jacket 40 via the inlet 38 and the outlet 37.

To precipitate chromosomal DNA, proteins and cell debris from the content of the reaction chamber, predetermined amount of the precipitating solution from the precipitating solution container 17 is, then, introduced into the rection chamber using upward movement of the piston 36 (Fig.3, D) via the tubing 18, the multiport valves 4 and the tubing 6 (39).

The mixture is agitated using the agitating means 2 and, optionally, the reaction chamber is cooled by means of temperature control means 3. This is achieved by introduceing cooling liquid into the jacket 40 via inlet 38 and the outlet 46.

The contents of the reaction chamber 33 is then filtered through the filter 31 by means of pressure caused by downward movement of the piston 36 (Fig.3, E). A predetermined volume of the liquid content of the reaction chamber 33 is collected by the loop 22 via the tubing 8 (43) and the multiport valves 7, while precipitated chromosomal DNA, proteins and cell debris will remain on the filter 31.

To purify extra-chromosomal DNA, the contents of the loop 22 is applied to the chromatographic column 26 by means of the pump 11. Impure fractions are supplied to waste by the valve 19, while pure extra-chromosomal DNA is collected in the vial 30 from the drip nozzle 28.

At the same time as the chromatographic purification takes place, the precipitate remaining on the filter 31 and within the reaction chamber 33, is dissolved in that a predetermined amount of the dissolving and cleaning solution from the container 23 is introduced by upward movement of the piston 36 (Fig.3, F) into the reaction chamber 33 through the tubing 25, the multiport valves 4 and the tubing 6 (39).

After agitating the reaction chamber 33 by means of the agitating means to dissolve and loosen all the precipitate and, optionally by heating the chamber by introducing heating water into the jacket 40 using the inlet 38 and the outlet 37, the dissolved precipitate is supplied to waste by means of the pressure caused by downward movement of the piston 36 (Fig 3, G) via the tubings 6 (39), the multiport valves 4 and the tubing 16.

This procedure can be repated one (Fig.3, H and I) or several times (not shown). The reaction chamber 33 is then ready to receive another cell suspension from the sampler 21 to start a new purification cycle.

Instead of the column 26, means can be provided to add a DNA precipitating agent to the contents of the loop 22 to purify extra-chromosomal DNA by precipitation in a manner known per se.

The means for purifying extra-chromosomal DNA may also comprise adsorbing means, e.g. a solid adsorption matrix, for adsorbing purified extra-chromosomal DNA from the contents of the loop 22.

The purification mentioned in the above two paragraphs is suitably carried out in a separate chamber or container (not shown).

CLAIMS

1. Apparatus for automated purification of extra-chromosomal DNA from a cell suspension using a reaction device (1) comprising

- means (36,21,4,5,6,34,39) for introducing the cell suspension from a container (20) into a reaction and/or mixing chamber (33),
- means (2) for agitating the contents of the chamber (33),
- means (36,14,15,4,6,39) for introducing a lysing solution into the chamber (33) to lyse the cells therein,
- means (36,17,18,4,6,39) for introducing a precipitating solution into the chamber (33) to precipitate chromosomal DNA and, optionally, proteins and cell debris therein,
- means (36,7,8,43) for feeding the liquid contents of the chamber (33) through a filter (31) to a collecting device (22),
- means (26) for purifying extra-chromosomal DNA from the contents of the collecting device (22),
- means (36,25,23,24,4,5,7,8,43) for introducing a dissolving solution into the chamber (33) to dissolve the precipitate remaining therein, and
- means (5,6,39,34,4,16) for feeding the dissolved precipitate to waste.

2. Apparatus according to claim 1, wherein the chamber (33) comprises said filter (31).

3. Apparatus according to claim 1, wherein a temperature control means (3) is provided to raise the temperature of the chamber (33) during lysing of the cells, and lower the temperature of the chamber (33) during the precipitation of chromosomal DNA and, optionally, proteins and cell debris.

4. Apparatus according to claim 1, wherein said reaction device (1) and pump means (32) are provided to control the flow of liquids within the apparatus.

5. Apparatus according to claim 1, wherein said collecting device comprises a loop (22) of predetermined or variable volume.

6. Apparatus according to claim 1, wherein said means for purifying comprises a chromatographic gel filtration column (26) connected to an output of the collecting device (28) and means (28,30) for collecting extra-chromosomal DNA containing fractions eluted from the column (26).

7. Apparatus according to claim 6, wherein a means (36,25, 23,24,7,8,22,27) is provided for introducing a cleaning solution into the column (26) to clean it.

8. Apparatus according to claim 1, wherein said means for purifying comprises means for adding a DNA precipitating agent to the contents of the collecting device (22) to precipitate extra-chromosomal DNA.

9. Apparatus according to claim 1, wherein said means for purifying comprises means for adsorbing purified extra-chromosomal DNA from the contents of the collecting device (22).

10. Apparatus according to claim 1, wherein said means for adsorbing further comprises a solid adsorption matrix.

11. Method for automatic purification of extra-chromosomal DNA from a cell suspension using a reaction device, comprising

- introducing the cell suspension by suction created in the reaction device into a reaction and/or mixing chamber,
- introducing a lysing solution by said suction into the chamber and mixing it with the contents thereof to lyse the cells therein,
- introducing a precipitating solution into the chamber and mixing it with the contents thereof to precipitate chromosomal DNA and, optionally, proteins and cell debris therein,
- filtering the contents of the chamber through a filter and feeding the liquid contents to a collecting device,
- purifying extra-chromosomal DNA from the contents of the collecting device,
- introducing a dissolving solution into the chamber and

mixing it with the contents thereof to dissolve the precipitate remaining therein, and
- feeding the dissolved precipitate from the chamber to waste.

12. Method according to claim 11, comprising raising the temperature of the chamber during the lysis of the cells, and/or optionally lowering the temperature of the chamber during the precipitation of the chromosomal DNA and, optionally proteins and cell debris.

13. Method according to claim 11, comprising using vacuum and/or pressure provided by said reaction device and pressure from pump separated from the reaction device to control the flow of liquids.

14. A reaction device capable of being used in said apparatus and method for automatic purification of extra-chromosomal DNA from a cell suspension and comprising
- a reaction and/or mixing chamber (33) having a volume determined by the position of a piston (36) movable inside said chamber (33),
- at least one filter (31) provided in the bottom of the chamber (33) on a filter support and sealing gasket (31),
- at least one inlet/outlet port connected to a lower part of the chamber (33) via a tubing (39) while an outlet port is connected via a tubing (34) to the upper part of the chamber (33) and preferably through the piston (36), and
- at least one inlet/outlet port at the bottom of the reacion device (1) below the filter (31) connected to the chamber (33) via a tubing (43).

15. A device according to claim 14, wherein the chamber (33) is surrounded by a space jacket (40) provided to supply heating/cooling liquids in order to control the temperature in said chamber (33).

16. A device according to claim 14, wherein the piston (36) is connected to a stepping motor (32) by a piston rod (41) with a thread for transfering a rotating movement to an up- and downward movement of the piston (36).

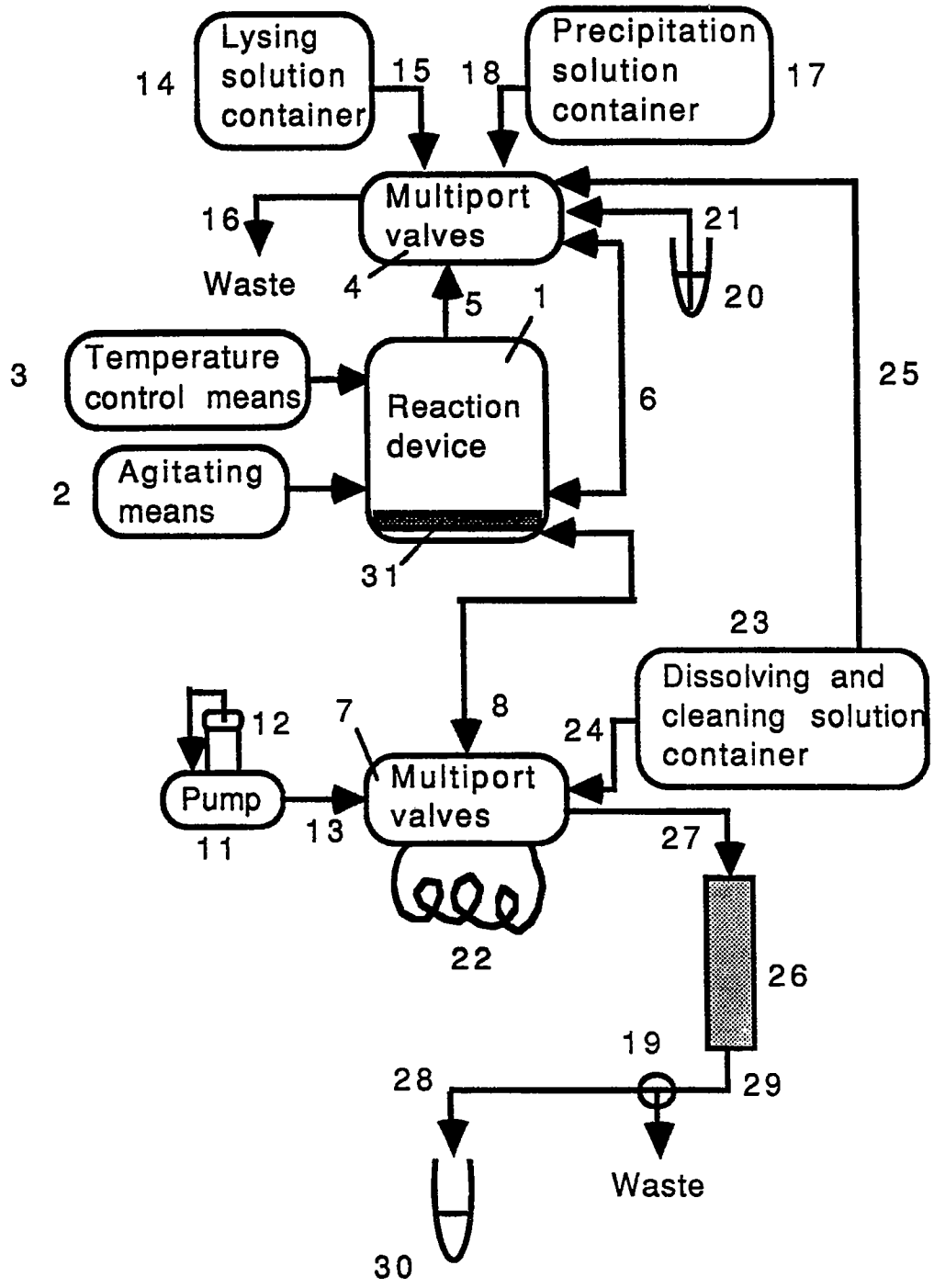
Lysing solution container
14
15
18
Precipitation solution container
17
Multiport valves
16
Waste
4
5
1
21
20
3
Temperature control means
Reaction device
6
25
2
Agitating means
31
23
Dissolving and cleaning solution container
12
7
8
24
Pump
11
13
Multiport valves
27
22
26
19
28
29
Waste
30

Figure 1

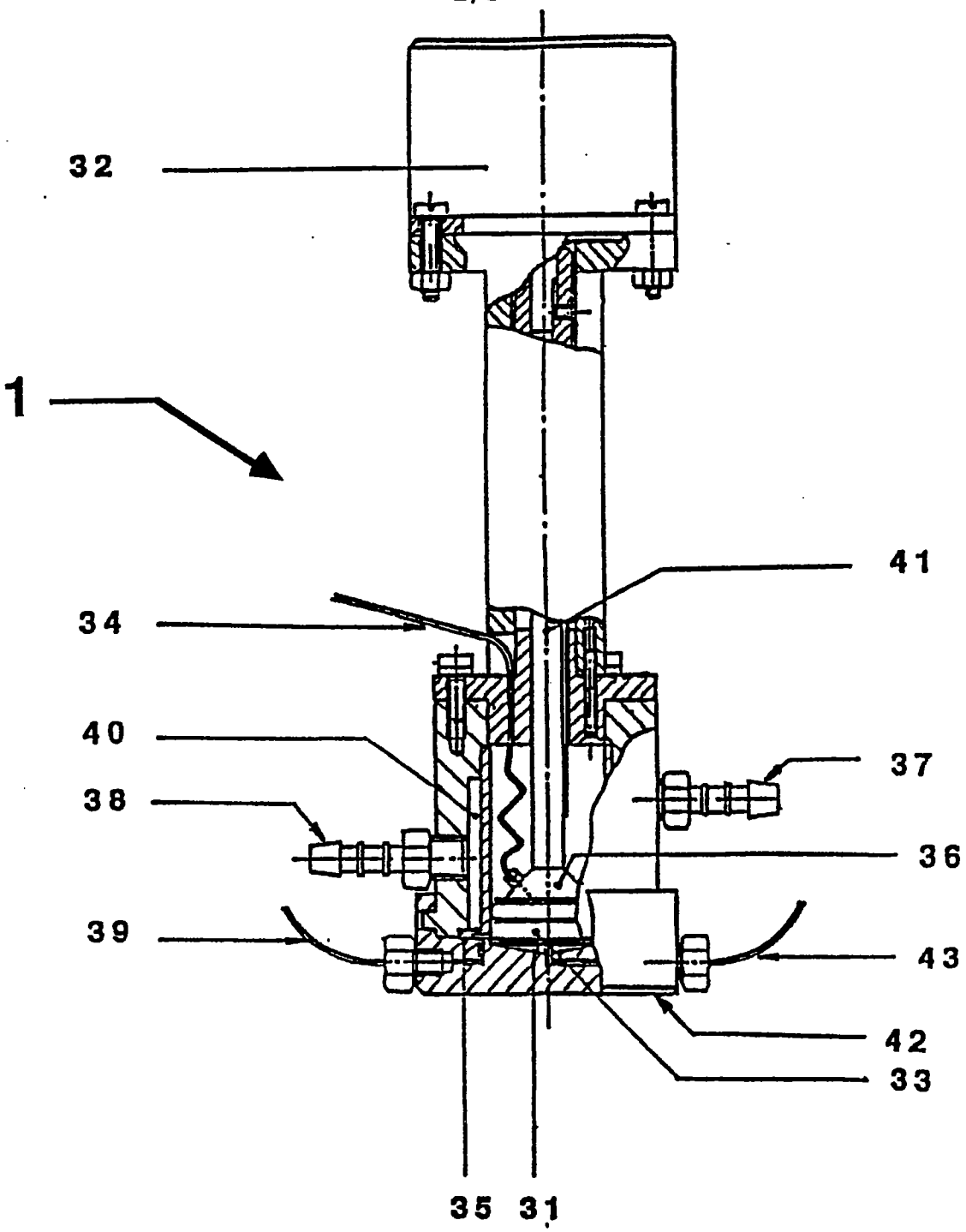

Figure 2

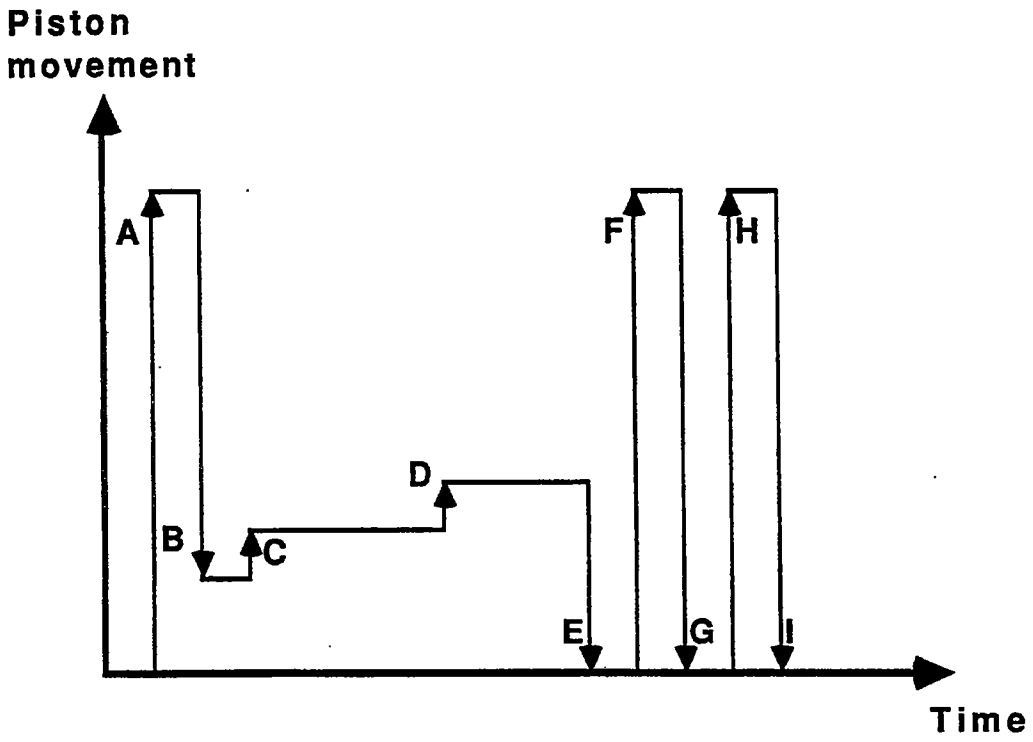

Figure 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 89/00313

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 P 19/34, C 12 N 15/00, C 12 M 1/00 //G 01 N 35/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC5	C 12 M; C 12 N; C 12 P; G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4833239 (D A DE BONVILLET ET AL) 23 May 1989, see column 3, line 10 - column 4, line 7 --	1-15
Y	EP, A2, 0215533 (APPLIED BIOSYSTEMS INC) 25 March 1987, see page 4 line 25 - page 6 line 10, claims --	1-15
Y	EP, A2, 0245945 (APPLIED BIOSYSTEMS INC) 19 November 1987, see pages 4-6, claims --	1-15
Y	GB, A, 2130744 (LABORATORIUM PROF. DR. RUDOLF BERTHOLD) 26 October 1983, see column 1, line 33 - line 58; abstract --	1,4,11, 13,14, 16

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
2nd January 1990	1990 -01- 12
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Niklas Forslund

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Chemical Abstracts, volume 110, no. 5, 30 January 1989, (Columbus, Ohio, US), W F Lima et al : "An efficient preparative scale plasmid purification method ", see, abstract 37725f, & Am.Biotechnol.Lab. 1988, 6(8), 20- 2 --	1-15
Y	Analytical Biochemistry, Vol. 148, 1985 D Micard et al: "Purification of RNA-free plasmid DNA using alkaline extraction followed by ultrogel A2 column chromatography ", see page 121 - page 126 --	1-15
Y	Preparative Biochemistry, Vol. 15, No. 3, 1985 J M Ranhand: "The enrichment of plasmid DNA:s, in bacterial cell lysates, using an alkaline-pH procedure that does not permanently damage them ", see page 121 - page 131 --	1-15
Y	Chemical Abstracts, volume 100, no. 5, 30 January 1984, (Columbus, Ohio, US), L. Just et al : "Rapid high-yield purification of plasmids by alkaline extraction, PEG precipitation and agarose gel chromatography ", see, abstract 31842p, & BioTechniques 1983, 1(3), 136-1380 --	1-15

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/SE 89/00313

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4833239	23/05/89	NONE	
EP-A2- 0215533	25/03/87	JP-A- 61167697	29/07/86
EP-A2- 0245945	19/11/87	JP-A- 63022194	29/01/88
GB-A- 2130744	26/10/83	FR-A- 2535459	04/05/84
		DE-A- 3239866	03/05/84